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ORIGINAL ARTICLE

Stereology study of oral verrucous carcinoma

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Summary

Purpose: *Squamous cell carcinoma (SCC) is the most prevalent malignant neoplasm of the oral cavity and oral verrucous carcinoma (OVC) is a verrucous variant of SCC. The purpose of this study was to examine the clinical classification of OVC and see for any difference in the biological behavior between OVC and CSS.*

Methods: *OVC and SCC were divided into 5 groups: the exogenic type of OVC (eOVC), cystoid type of OVC (cOVC) and infiltrative type of OVC (iOVC); well differentiated SCC (wdSCC), and medium/poorly differentiated SCC (m/pdSCC). A normal mucosa (NM) group was also created and studied. Stereology was used to measure and describe the morphological parameters of the nucleus to cytoplasm ratio (Vnp), desmosomes, mitochondria, etc. Analysed were also the nucleus volume density (Vv), Vnp, desmosomes and intracellular desmosomes number density (Nv), which were observed by stereology.*

Results: *We noticed some statistically significant differences in the morphological parameters among the 6 groups including the Vv ($p<0.05$), the Vnp ($p<0.05$), the number density of desmosomes ($p<0.05$), and the Nv ($p<0.05$).*

Conclusion: *This study provides a theoretical basis for the clinical diagnosis and therapy of OVC.*

Key words: oral verrucous carcinoma, squamous cell carcinoma, stereology, ultrastructure

Introduction

Cancer is an important global public health problem, and oral cancer is one of the 10 most common cancers worldwide [1]. Among oromaxillofacial region tumors, SCC is the most prevalent malignancy. OVC is a verrucous variant of SCC, which has aroused wide concern.

OVC is a rare tumor first described by Ackerman in 1948 [2]; since then, this tumor has gradually attracted considerable attention. The tumor occurs in the elderly, has papillary appearance, and grows slowly and locally during several years [3-7]. It is well differentiated, metastasizes only rarely, and has good prognosis; however, it is difficult to be cured after recurrence, and some cases may be misdiagnosed for odontogenic keratocysts and periodontitis [8]. Tang et al. proposed that the OVC of the same or similar pushing border structure displays 3 clinical classifications and ultrastructures in light microscopy: eOVC, cOVC and iOVC. OVC is a malignant tumor with its own independent biological characteristics, and is different from oral squamous cell carcinoma (oSCC) [8]. It has been proved that the occurrence, development and prognosis of OVC are related to CK, CD44v6, Ki67, c-erbB-2, cathepsin, p53, MMPs, Moesin, and OPN genes [9-12].

A stereological method called "three dimension studies" is widely used to measure quantitative changes in tissues. The method used to determine the volumes of various subdivisions of organs is based on the Cavalieri principle. According to this principle, the volume of arbitrary complex structures can be estimated from the sum of parallel areas separated by a known distance, provided that the set of the sections is positioned randomly with respect to the chosen axis [13].

OVC has been studied clinically and morphologi-

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cally, and also described ultrastructurally; however, no quantitative detection and description of 3 clinical classification of OVC have been carried out so far. In our study, we used stereology to estimate the 3 clinical classification of OVC, and the morphological parameters Vv, Vnp, desmosomes and Nv, from OVC, wdSCC, m/pdSCC, and from normal tissues, and discuss the organelle variation of OVC and SCC in order to prove that the 3 clinical classifications of OVC and the difference in biological behavior between OVC and SCC could provide a theoretical basis for the diagnosis and treatment of OVC.

Methods

Specimen source

Thirty cases in well-preserved paraffin-embedded specimens of oral cancer were acquired from the Department of Oral Pathology (Hospital of Stomatology, Central South University, 1992-2008). There were 5 cases in each of the following groups: eOVC, iOVC, cOVC, wdSCC, m/pdSCC, and NM as the control group. No patient was subjected to radiotherapy and chemotherapy before operation. OVC clinical classification was done according to Tang et al. system [8]. The clinical patient data are shown in Table 1.

Sample preparation

For histological analysis, all samples were fixed by immersion in Bouin's and 10% formaldehyde, embedded in paraffin, cut into 5-μm thick sections and stained with hematoxylin and eosin. The slides were photographed with an Olympus photomicroscope.

For ultrastructural analysis, 5 tissue paraffins blocks from each experimental group containing cancerous tissue epithelium near the basement membrane were stained with hematoxylin and eosin. Next, epithelia near the basement membrane were chosen and cut into small pieces < 1 mm^3. Then, all the material was immersed in 100% xylene, anhydrous acetone, epoxy resin acetone mixture (embedding liquid: anhydrous acetone =1:1) and embedded in resin, cut with an ultramicrotome, mounted on copper grids, and counterstained with uranyl acetate and lead citrate. Thin sections were collected on uncoated 200-mesh copper grids and viewed with a JEOL-1230 electron microscope operating at 80 KV.

Transmission electron microscopy

Five random images were taken for transmission electron microscopy (TEM) at ×5,000 and ×50,000 magnification. One photomicrograph represented one sample according to the stereological principle, so the number of samples of each group was 5 × 5 = 25 samples.

Observation index and calculating methods

At ×10,000 TEM magnification the morphological characteristics of tissue ultrastructure were studied. The observation index of stereology included Vv, Vnp, desmosomes and Nv. Observation and statistics of the nucleus and the cytoplasm were made via ×5,000 magnification electron micrographs, while observation and statistics of desmosomes and Nv were made via ×10,000 magnification electron micrographs.

Unbiased estimates of the Vv, Nv and cytoplasmic desmosomes, and the ratio of nucleus to nucleolus were obtained according to the method developed by Gundersen and Jensen [14]. This method is based on point-sampling. For the Vv parameters the following formula was used:

$$Vv=Pxn/Prn,$$

where Pxn was the number of points hitting the histological structures and Prn was the total number of points hitting the reference histological space.

To estimate the number density of desmosomes/cytoplasmic desmosomes parameters, the following formula was used:

$$Nv=Nt/Vr,$$

where Nt was the number of desmosomes or cytoplasmic desmosomes in the histological structures and Vr was the total number of points hitting the reference histological space.

The nucleus to cytoplasm ratio parameters were calculated using the following formula:

$$Vnp=Pn/Pp,$$

where Pn was the number of points hitting the nucleus and Pp was the total number of points hitting the cytoplasm of the same cell.

To ensure that points and organelles were not overcounted, these were not counted if any point touched the left and front surfaces of the cell membrane.

Statistical considerations

The measurement of all data was double-blind. Values were calculated as mean ±standard error of the mean (SEM) and ± standard deviation. Data were analysed using one-way analysis of variance (ANOVA). Normality and homogeneity of the variances were checked using the Levene test before ANOVA. Significant differences among treatments were calculated by the LSD test (least sig-

Table 1. Clinical data

Disease groups	*No. of cases*				*Localization*					
		M	*F*	*Age, years ±SD*	*Tongue*	*Cheek*	*Lip*	*Jaw bones*	*Gum*	*Pars palatalis*
NM	5	3	2	30.7±5.3	0	3	0	0	2	0
eOVC	5	4	1	53.0±12.4	0	3	2	0	0	0
iOVC	5	5	0	48.0±11.5	0	1	0	1	3	0
cOVC	5	2	3	52.0±13.7	0	0	0	4	1	0
wdSCC	5	3	2	54.0±14.2	3	1	0	0	1	0
m/pdSCC	5	2	3	49.0±12.3	0	1	1	0	2	1
Total	30	19	11	47.8±8.6	3	9	3	5	9	1

M: male, F: female, SD: standard deviation. For disease groups abbreviations see text

nificant difference) and a p value <0.05 was considered as statistically significant.

Results

Morphological observation

NM group

Regular, embedded and tightly arranged cells were observed; small and regular-shape nuclei and small nucleoli were also detected; and large ratio of the cytoplasm, lots of desmosomes between cells, few intracytoplasmic desmosomes, abundant mitochondria and endoplasmic reticulum were visible.

eOVC group

Epithelial cells were well differentiated, intercellular space was slightly wider than NM, cell borders were clear, with no significant pseudopods on the membrane and more regular-shaped cells were observed. Keratin pearls could be seen in epithelial cells; large and regular nucleus with nucleoli and little chromatin were visible; the ratio of cytoplasm was smaller than that of NM, lots of desmosomes but few in the cytoplasm and a few mitochondria were also observed (Figures 1A and 2A).

cOVC group

Epithelial cells were poorly differentiated, atypia was obvious, cells showed slender spindle shape, intercellular space was wider than that of eOVC, cell boundaries were clear and desmosomes between cells were fewer, while pseudopodia could be seen on the cell membrane; meanwhile, we observed large nuclei which were irregularly shaped, notched and lobulated; a small proportion of the cytoplasm had vacuoles change, many intracytoplasmic desmosomes, a few mitochondria and endoplasmic reticulum (Figures 1B and 2B).

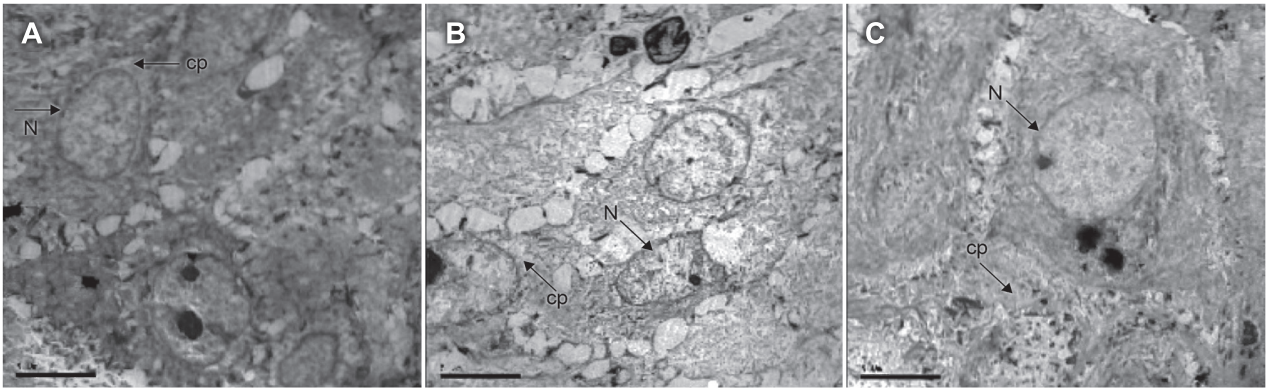

Figure 1. A. Large and regular nuclei (N) with notch and small ratio of cytoplasm (CP) in the eVOC group (×5000). **B.** Irregular and spindle nuclei (N) with notch and small ratio of cytoplasm (CP) in the cVOC group (×5000). **C.** Large and irregular nuclei (N) with notch and small ratio of cytoplasm (CP) in the iVOC group (×5000).

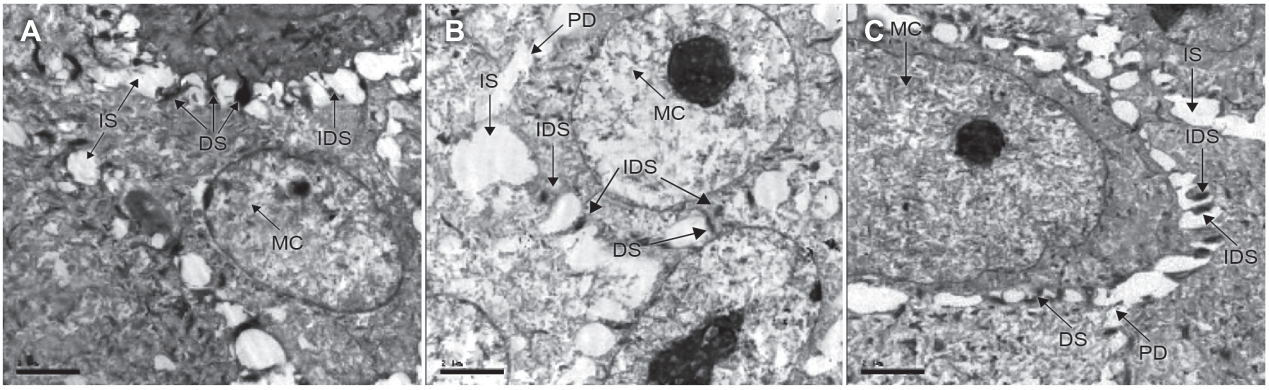

Figure 2. A. Mitochondria (MC); the intercellular space (IS) is widened slightly in the eVOC group (×10000). **B.** There are a few desmosomes (DS), many intracytoplasmic desmosomes (IDS) and mitochondria (MC) in the cVOC group, while the intercellular space (IS) is widened with pseudopods (PD) (×10000). **C.** There are a few desmosomes (DS), many intracytoplasmic desmosomes (IDS) and few mitochondria (MC) in the iVOC group, while the intercellular space (IS) is widened with pseudopods (×10000).

iOVC group

We could see poorly differentiated epithelial cells, obvious atypia, wider intercellular space than eOVC, clear cell boundaries, fewer desmosomes, lots of pseudopodia on the cell membrane, and stress fibers intruded into pseudopods along the membrane and connected with desmosomes; we also found large nuclei which had irregular shape, were notched, lobulated and depressed; chromatin was abundant near the nucleoli, obviously, more chromatin were seen near the membrane; a small proportion of the cytoplasm which had vacuoles change were observed, and some cells only been kept naked nuclei; many intracytoplasmic desmosomes, a few mitochondria and endoplasmic reticulum were visible (Figures 1C and 2C).

wdSCC group

Cancer cells were bulky and irregular in shape, cell boundaries were clear, intercellular space was slightly widened, no pseudopodia were seen surrounding the cell membrane; nuclei were large, irregularly shaped and notched; we also found that the cytoplasm occupied a small proportion, nuclear euchromatin was rich, and nucleoli were prominent, more chromatin was seen near the nuclear membrane, desmosomes were abundant, but intracytoplasmic desmosomes and mitochondria were less and keratohyalin in granules could be seen in the cytoplasm.

m/pdSCC group

Cancer cells were small and irregular in shape, intercellular space was widened, and cells were almost free, nuclei were large, irregularly shaped and notched; the cytoplasm occupied a small proportion, desmosomes and intracytoplasmic desmosomes were less and so were mitochondria, endoplasmic reticulum and other organelles.

The average and standard deviation of the nuclear volume density in groups NM, eOVC, cOVC, iOVC, wdSCC, m/pdSCC are shown in Table 2.

These results showed that there were significant differences about the nuclear volume density of NM, eOVC, cOVC, iOVC, wdSCC and m/pdSCC ($p<0.05$). Pairwise comparison showed: 1) three OVC groups: the nuclear volume density of eOVC was significantly lower than that of iOVC ($p<0.05$); there were no significant differences between any of the other two groups; 2) the nuclear volume density of eOVC, cOVC, iOVC, wdSCC and m/pdSCC were significantly higher than that of NM ($p<0.05$); the nuclear volume density of m/pdSCC was significantly higher than that of other groups ($p<0.05$); the nuclear volume density of cOVC and iOVC was significantly higher than that of wdSCC ($p<0.05$); there was no statistically significant difference between the nuclear volume density of eOVC and wdSCC.

Average and standard deviation of the nucleus to cytoplasm ratio in groups NM, eOVC, cOVC, iOVC, wdSCC, m/pdSCC are shown in Table 3.

There were significant differences in the nucleus to cytoplasm ratio of NM, eOVC, cOVC, iOVC, wdSCC and m/pdSCC ($p<0.05$). Pairwise comparison showed: 1) three OVC groups: the nucleus to cytoplasm ratio of cOVC and iOVC were significantly higher than that of eOVC ($p<0.05$); there was no significant difference between the nucleus to cytoplasm ratio of cOVC and iOVC; 2) the nucleus to cytoplasm ratio of eOVC, cOVC, iOVC, wdSCC and m/pdSCC were all significantly higher than that of NM ($p<0.05$); there was no significant difference between the nucleus to cytoplasm ratio of eOVC and wdSCC; the nucleus to cytoplasm ratio of m/pdSCC was significantly higher compared with any other group ($p<0.05$); the nucleus to cytoplasm ratio of cOVC and iOVC was significantly higher than that of other groups ($p <0.05$).

The average and standard deviation of the number density of cytoplasmic desmosomes in groups NM, eOVC, cOVC, iOVC, wdSCC, m/pdSCC are shown in Table 4.

There were significant differences in the number density of cytoplasmic desmosomes of NM, eOVC, cOVC, iOVC, wdSCC and m/pdSCC ($p<0.01$). Pairwise comparison showed: 1) three OVC groups: the number density of cytoplasmic desmosomes of eOVC was significantly higher than that of cOVC and iOVC ($p<0.01$); there was no significant difference between the number density of cytoplasmic desmosomes of cOVC and iOVC; 2) there were no significant differences among cOVC, iOVC and m/pdSCC ($p>0.05$); there were significant differences among the other groups

Table 2. Average and standard deviation of the nuclear volume density

Disease groups	*N*	*Density of nuclei ± SD*
NM	25	0.1839±0.0746
eOVC	25	0.4398±0.0489
cOVC	25	0.4673±0.0660
iOVC	25	0.4825±0.0569
wdSCC	25	0.4259±0.0759
m/pdSCC	25	0.5362±0.0607

Levene analysis: $p>0.05$; ANOVA analysis: $F= 91.653$, $p<0.05$; LSD analysis: NM, eOVC, cOVC, iOVC, wdSCC, m/pdSCC: $p<0.05$; m/pdSCC, eOVC, cOVC, iOVC, wdSCC: $p<0.05$; eOVC and iOVC: $p<0.05$; cOVC and wdSCC: $p<0.05$; iOVC and eOVC, wdSCC: $p<0.05$

Table 3. Average and standard deviation of the nucleus to cytoplasm (N/C) ratio

Disease groups	*N*	*N/C ratio±SD*
NM	25	0.2262±0.1286
eOVC	25	0.7761±0.1677
iOVC	25	0.9070±0.2548
cOVC	25	0.9319±0.2861
wdSCC	25	0.7508±0.2220
m/pdSCC	25	1.2196±0.2082

Levene analysis: p>0.05; ANOVA analysis: F= 57.24, p<0.05; LSD analysis: NM and eOVC, cOVC, iOVC, wdSCC, m/pdSCC: p<0.05; eOVC: exogenic type of oral verrucous carcinoma; wdSCC: well differentiated squamous cell carcinoma; cOVC: cystoid type of oral verrucous carcinoma; iOVC: infiltrative type of oral verrucous carcinoma; SD: standard deviation

Table 5. Average and standard deviation of the number density of cytoplasmic desmosomes

Disease groups	*N*	*Nv±SD*
NM	25	0.0092±0.0141
eOVC	25	0.0141±0.0121
iOVC	25	0.0388±0.0159
cOVC	25	0.0388±0.0211
wdSCC	25	0.0145±0.0249
m/pdSCC	25	0.0242±0.0218

Nv: density of desmosomes; Levene analysis: p>0.05; ANOVA analysis: F=65.9, p<0.05; LSD analysis: NM, cOVC, iOVC and m/pdSCC: p<0.05; eOVC, cOVC and iOVC: p<0.05; there were no significant differences among NM, eOVC, wdSCC, and moreover there were no significant differences among cOVC and iOVC; SD: standard deviation

(NM>eOVC>wdSCC>cOVC, iOVC and m/pdSCC; p <0.05).

The average and standard deviation of the number density of cytoplasmic desmosomes in groups NM, eOVC, cOVC, iOVC, wdSCC, m/pdSCC are shown in Table 5.

There were significant differences in the number density of cytoplasmic desmosomes of NM, eOVC, cOVC, iOVC, wdSCC and m/pdSCC (p<0.05). Pairwise comparison showed: 1) three OVC groups: the number density of cytoplasmic desmosomes of eOVC was significantly lower compared with cOVC and iOVC (p<0.01); there was no significant difference between the number density of cytoplasmic desmosomes of cOVC and iOVC; 2) the number density of cytoplasmic desmosomes of cOVC and iOVC was significantly higher than that of any other group (p<0.05); no significant differences were seen in the number density of cytoplasmic desmosomes of NM, eOVC and wdSCC (p>0.05); the number density of cytoplasmic desmosomes of m/pdSCC was significantly higher than that of NM, eOVC and wdSCC (p<0.05).

Discussion

OVC has been distinguished from SCC as an independent entity and its morphological study was focused on the examination of its structure under light microscopy. The most typical feature is that all epithelial processes infiltrate the connective tissue in almost the same depth, which Ackerman called "pushing border" [2]. However, the structure under light microscopy cannot explain the diversity of the biological behavior of clinical OVC. Another study [15] showed that OVC had 3 ultrastructural types, however this morphological description was based on the general ultrastructure of OVC.

In our study we observed the ultrastructural differences of 3 types of the OVC clinical classification. It was proved that pseudopod formation on the membrane was influenced by mitogenic factors and was enhanced following malignant transformation [16], leading to cell surface microvilli, which indicated that pseudopod formation was related to tumor invasion. It was also observed that lack of connections, intimate contact and gap junctions, and the number of desmosomes and hemidesmosomes decreased between invasive cancer cells. In the eOVC group the intercellular space was slightly wider than that in the NM group; meanwhile, we observed clear cell boundary, regular cell shape, keratin pearls in epithelial cells, large and regular nucleus shape with nucleolus and less chromatin, lower ratio of the cytoplasm vs. total cell volume with few desmosomes and mitochondria. All these features implied that cells were well differentiated and atypia was less in the eOVC group, while no pseudopod formation on the membrane implied low invasive potential and good biological behavior.

In cOVC and iOVC groups we observed irregular cell shape and few desmosomes, while lots of pseudopods appeared on the cell membrane, and stress fibers intruded into pseudopods along the membrane and connected with desmosomes; large and irregular nuclei were also observed, which had a notch, were depressed and lobular, with obvious nucleoli and rich in chromatin with more chromatin observed near the nuclear membrane. The ratio of cytoplasm vs. total cell volume was

Table 4. Average and standard deviation of the number density of desmosomes

Disease groups	*N*	*Density of desmosomes ± SD*
NM	25	0.0729±0.0176
eOVC	25	0.0444±0.0190
iOVC	25	0.0129±0.0156
cOVC	25	0.0088±0.0103
wdSCC	25	0.0283±0.0159
m/pdSCC	25	0.0058±0.0231

Levene analysis: p>0.05; ANOVA analysis: F=65.9, p<0.05; LSD analysis: NM and eOVC, cOVC, iOVC, wdSCC, m/pdSCC: p<0.05; cOVC, iOVC, m/pdSCC: p>0.05; other groups: p<0.05

small, with vacuolated changes, and only naked nuclei in some cells; lots of desmosomes and few mitochondria and endoplasmic reticulum were also observed in the cytoplasm, revealing the difference of biological behavior between cOVC and iOVC, and connecting these features with the clinical biological behavior. However, there wasn't statistically significant difference between cOVC and iOVC, and spindle cells were observed only in the cOVC group, but the specific reasons are unclear.

It has been proved that the abnormally enlarged nucleus is related to the progression and prognosis of many tumors, e.g. thyroid cancer, colon cancer, breast cancer, oral squamous cell carcinoma etc. [17,18], and the shape and size of the nucleus can reflect the cell's degree of differentiation and its biological behavior. Firstly, in our study, enlarged nuclei in oral squamous cell carcinoma were found. Secondly, the nuclear volume density of eOVC was significantly lower compared to iOVC. So, it can be speculated that the biological behavior of eOVC is better than that of iOVC, which is consistent with the biological behavior of clinical cOVC and iOVC. Thirdly, it was found that the nuclear volume density of cOVC and iOVC was higher than that of wdSCC, while there was no obvious difference between eOVC and wdSCC, indicating that the degree of differentiation of cOVC and iOVC was lower compared with wdSCC.

Vnp is the ratio of nucleus to cytoplasm area. Vnp usually increases after malignant transformation and rarely changes with benign lesions [19]. It is an important feature of tumor atypia, and the abnormal increase of Vnp is related to the progression and prognosis of many tumors, reflecting their degree of differentiation and biological behavior [20,21]. In this study, the Vnp of cOVC and iOVC was significantly higher than that of eVOC, and there was no obvious difference between cOVC and iOVC, indicating that the degree of the eOVC differentiation is better than that of cOVC and iOVC, and the biological behavior is similar between cOVC and iOVC. The Vnp of m/pdSCC was significantly higher than that of all other tissues; the Vnp of cOVC and iOVC was significantly higher than that of wdSCC and there was no significant difference between eOVC and wdSCC, indicating that the biological behavior of iOVC and cOVC is inbetween of wdSCC and m/pSCC behavior, while sometimes the biological behavior of cOVC and iOVC is even worse than that of m/pSCC.

Accumulating evidence shows that desmosomes might prevent tumor invasion and metastasis. The expression of desmoglein and desmoplakin is downregulated in some head and neck SCCs depending on poorly differentiated primary tumor, the degree of invasion and lymph node metastasis [22]. Tselepis et al. had demonstrated that desmosome adhesion could prevent tumor invasion *in vitro*, lending support to the view that tumor spread is inhibited by desmosomes [23]. Li et al. observed that simple connection and contact connection occupied the main position in cancer cells and desmosome connections reduced or even disappeared in tumors of peritoneal micrometastasis-positive patients using TEM, while the clusters of desmosomes connection appeared in tumor cells of negative patients [24]. In this study, we found that desmosomes decreased in OVC, wdSCC and pdSCC. The Nv of eVOC was higher than that of cVOC and iVOC, and while there was no significant difference between the Nv of cVOC and iVOC, we speculate that the biological behavior of eVOC is better than that of cVOC and iVOC. No obvious difference among cVOC, iVOC and m/pdSCC in the 6 groups was noted, however, statistically significant difference existed the eOVC, wdOSCC and NM groups. So, one can speculate that the invasion of cVOC, iVOC and m/pdSCC was rather small, and they were all highly malignant and poor prognosis tumors, which is consistent with the biological behavior of clinical cVOC and iVOC. At the same time, we found that the Nv of NM was higher compared with the other 5 groups, which also proved that desmosomes might prevent tumor invasion and metastasis.

The mechanism of desmosome formation is unclear. Some authors have stated that desmosome formation is produced momentarily according to the needs of cells after cells complete split [25]. However, most researchers believe that the encapsulated glomeration of cytoplasmic desmosomes reflects the degree of differentiation of malignant tumors [26]. In our study, there were no significant differences among NM, eVOC and wdSCC, which is in accordance with the source of intracellular desmosomes, indicating that eVOC and wdSCC were well differentiated and less invasive. The Nv of eVOC was significantly lower than that of cVOC and iVOC, which indicated that eVOC had better differentiation and prognosis than cVOC and iVOC, in correlation with the biological behavior and prognosis of the 3 clinical classifications of OVC, and provided ultrafine evidence for OVC classification.

Conclusion

a) We found that the 3 clinical classification of OVC had different ultrastructural basis. b) The nuclei, the nucleus to cytoplasm ratio, and the intracellular desmosomes of eOVC are significantly less than the ones of cOVC and iOVC; the desmosomes between cells of eOVC are obviously more than the ones of cOVC and iOVC. c) The grade of cell differentiation of iOVC and cOVC lies between wdSCC and m/pdSCC. d) Compar-

ing the intracellular desmosomes, the degree of differentiation of iOVC and cOVC is even worse compared with m/pdSCC.

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References

1. Scully C, Newman L, Bagan JV. The role of the dental team in preventing and diagnosing cancer: 2. Oral cancer risk factors. Dent Update 2005; 32: 261-270.
2. Ackerman LV. Verrucous carcinoma of oral cavity. Surgery 1948; 23: 670-678.
3. Ogawa A, Fukuta Y, Nakajima T et al. Treatment results of oral verrucous carcinoma and its biological behavior. Oral Oncol 2005; 41: 436-438.
4. Strojan P, Soba E, Budihna M et al. Radiochemotherapy with Vinblastine, Methotrexate and bleomycin in the treatment of verrucous carcinoma of the head and neck. J Surg Oncol 2005; 92: 278-283.
5. Oliveira MC, Silveira EJ, Godoy GP et al. Immunohistochemical evaluation of intermediate filament proteins in squamous papilloma and oral verrucous carcinoma. Oral Dis 2005; 11: 288-292.
6. Adegboyega PA, Boromound N, Freeman DH. Diagnostic utility of cell cycle and apoptosis regulatory proteins in verrucous squamous carcinoma. Appl Immunohistochem Mol Morphol 2005; 13: 171-177.
7. Impola U, Uitto VJ, Hietanen J et al. Differential expression of matrilysin-1 (MMP-7), 92 kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. J Pathol 2004; 202: 14-22.
8. Tang ZG, Xie XL, Li JY et al. A clinical study on oral verrucous carcinoma phenotypes. Chin J Dental Res 2005; 8: 57-61.
9. Zhang L, Tang ZG, Zhou ZG et al. The expression of Maspin and VEGF gene in oral squamous cell carcinoma and its significance. Shanghai Kou Qiang Yi Xue 2005; 14: 557-560 (in Chinese).
10. Tang ZG, Zou P, Xie XL. Expression of E-cadherin gene protein in oral verrucous carcinoma. Hunan Yi Ke Da Xue Xue Bao 2003; 28: 206-208 (in Chinese).
11. Tang ZG, Zhao SP, Zhang L, Li XL. Gene expression profile changes in oral verrucous carcinoma and oral squamous cell carcinoma. Zhonghua Kou Qiang Yi Xue Za Zhi 2007; 42: 229-230 (in Chinese).
12. Lin HP, Wang YP, Chiang CP et al. Expression of p53, MDM2, p21, heat shock protein 70, and HPV 16/18 E6 proteins in oral verrucous carcinoma and oral verrucous hyperplasia. Head Neck 2011; 33: 334-340. doi: 10.1002/hed.21452.
13. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229-263.
14. Gundersen HJ, Bendtsen TF, Korbo L et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988a; 96: 379-394.
15. Kannan S, Kartha CC, Chandran GJ et al. Ultrastructure of oral squamous cell carcinoma: a comparative analysis of different histological types. Eur J Cancer B Oral Oncol 1994; 30B: 32-42.
16. Wu ZB (Ed): Ultrastructural pathology diagnostics (2nd Edn). Shanghai Science and Technology Press, 2003, pp 77-79.
17. Wotberg WH, Street WN, Mangasarian OL. Computer derived nuclear features compared with axillary lymph node status for breast carcinoma prognosis. Cancer (Cancer Cytopathol) 1997; 81: 172-179.
18. Masahide L, Jianhui C, Shinichi O et al. Nuclear profiles of cancer cells reveal the metastatic potential of gastric cancer. J Pathol 2000; 192: 19-25.
19. Yang GH (Ed): Pathology (5th Edn). People's Health Press, 2001.
20. Lobo MV, Alonso FJ, Redondo C et al. Cellular characterization of epidermal growth factor-expanded free-floating neurospheres. J Histochem Cytochem 2003; 51: 89-103.
21. Bez A, Corsini E, Curti D et al. Neurosphere and neurosphere-forming cells: morphological and ultrastructural characterization. Brain Res 2003; 993: 18-29.
22. Hiraki A, Shinohara M, Ikebe T et al. Immunohistochemical staining of desmosomal components in oral squamous cell carcinomas and its association with tumour behaviour. Br J Cancer 1996; 73: 1491 -1497.
23. Tselepis C, Chidgey M, North A et al. Desmosomal adhesion inhibits invasive behavior. Proc Natl Acad Sci USA 1998; 95: 8064-8069.
24. Li ZX, Jia YT, Ma SM et al. Significance of NF-kB and MMP-9 expressions in peritoneal micrometastases of colorectal carcinoma. Chin J Cancer Prevent Treat 2008; 15: 1014-1017 (in Chinese).
25. Zheng M, Xie FK, Zheng GF. An Electron Microscopic Observation on the Formation of Cytoplasmic Desmosome from Some Carcinoma Cells. J Qilu Oncol 1998; 5: 169-170 (in Chinese).
26. Ghadially FN (Ed): Ultrastructural pathology of the cell and matrix (2nd Edn). Woburn, Mass, Buttereworths, USA, 1982, p 740.